Identification of Lactoferrin gene Polymorphism and its association with Mastitis incidence

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ABSTRACT

Present study was conducted in total of 350 cows of two major dairy breeds (Sahiwal and Karan Fries) with the aim to identify genetic variation in lactoferrin gene promoter and to study its association with incidence of mastitis. Polymorphism of bovine lactoferrin gene promoter was determined by using restriction fragment length polymorphism (PCR-RFLP). Lactorferrin gene promoter was polymorphic but showed varied level of polymorphism among Sahiwal and Karan Fries cattle. Three genotypes were identified viz. AA, AB and BB in Karan Fries cattle and two genotypes AA and AB in Sahiwal cattle. BB genotype was absent in Sahiwal herd of National Dairy Research Institute, Karnal. Chi square test revealed a non-significant association ($pd \leq 0.05$) with mastitis incidence.

Keywords: Lactoferrin Gene, RFLP-PCR, Mastitis, Sahiwal, Karan Fries

Lactoferrin is a glycoprotein with molecular weight 80 kilo Dalton. It is a member of transferrin family capable of binding and transferring Fe^{3+} ions (Metz-Boutique *et al*. 1984). Lactoferrin is secreted from the exocrine gland and in specific granules of neutrophils in bovines. Lactoferrin is involved in many biological functions and is classified as acute phase protein (Kanyshkova *et al*. 2001) due to the increase in its concentration during most inflammatory reactions. Lactoferrin is one of the proteins present in bovine milk, which exerts several functions related to innate immunity and host defence.

Mastitis is an inflammatory condition of mammary glands in dairy cattle. Mastitis is one of the major production related disease in our country. According to an estimate loss due to mastitis approach $2 billion annually in the United States alone (Halloran, 2009) and about Rs 6053.21 crores in India (Burman, 2002). There are reports which suggest that Lactoferrin (Lf) concentration in milk and serum would change during the infection of mastitis (Barkema, 1998, Hirvoen, 1999), which indicated

that there was some association between Lactoferrin and mastitis. Therefore it is imperative to screening the Lactoferrin gene by RFLP, and to study association between Lactoferrin and mastitis incidence.

MATERIALS AND METHODS

Genomic DNA isolation and Polymorphism detection

The genomic DNA was isolated from aseptically collected venous blood from Sahiwal (n=200) and Karan Fries cattle (n=150) maintained at cattle yard of National Dairy Research Institute, Karnal. Phenol:chloroform method as suggested by Sambrook and Russel (2001) was used to isolate the genomic DNA.

Primer Designing

NCBI sequence accession number AY 319306 was used to design the primer. Forward and reverse primers (PF 5'- GATAAAGGGACGCAGAACGAGC-3' & PR 5'- ATACCTGCACTCACCAACGGCTCC -3') with T_M of 62.1°C for P_F and 66.1 °C for P_R, were designed using Primer-3 software, freely available online

PCR-RFLP

PCR amplification results a fragment size of 125 bp spanning over promoter region including exon1 of Lactoferrin gene. A total of 350 cattle were screened for the presence of single nucleotide polymorphism. PCR optimization was done to get the best possible amplification of the product, at an annealing temperature of 59.5^{0}C for one minute. The detection of results of allelic variation at SNP sites were based on the electrophoretic pattern of the restriction enzyme treated PCR products. *Sty*I restriction enzymes selected from MBI Fermentas, India and New England Biolabs, was used for RFLP analysis at 37°C for 8 hours in both the breeds. Selected PCR-RFLP products of different genotypes were sequenced using the automated dye terminator cycle sequencing method and sequence comparison was made using CLUSTAL W software.

Mastitis Incidence, Statistical and Genotype Analysis

Data on clinical mastitis was collected for all the screened cows were recorded from the treatment registers from organized herd of National Dairy Research Institute for the period of twelve year (2000 to 2012), and were used for further analysis. Genotype and allele frequencies were calculated and compared by gene counting method as suggested by Falconer and Mackay (1996). Association of Lactoferrin variants with affected and non-affected cows were calculated using Chi square Test (JMP 5.0, a business unit of SAS Copyright © 1989 - 2002 SAS Institute Inc.)

RESULTS AND DISCUSSION

Variations in Lactoferrin gene especially in promoter region could play an important role in the transcription and regulation Lf gene function. PCR-RFLP of bovine

Lactoferrin gene revealed three different genotypes namely, AA, AB and BB in Karan Fries cattle whereas the BB genotype was absent in Sahiwal cattle. In Karan Fries cattle AA, AB and BB genotypes were having a frequency 18.67%, 76.66% and 4.67% respectively. The frequency of A and B allele in Karan Fries cattle was found to be 0.57 and 0.43 respectively. In Sahiwal cattle AA and AB genotypes were found with genotype frequency 61% and 39% respectively. BB genotype was absent in Sahiwal cattle. The frequency of A and B allele in Sahiwal cattle were found to be 0.805 and 0.195 respectively.

Our results in Sahiwal cattle shows marked variations with Huang *et al*. (2010) and does not agree with respect to these alleles in Chinese Holstein Friesian cattle where they reported A and B alleles with 0.556 and 0.444 respectively. The marked variation may be attributed to the absence of BB genotype in Sahiwal cattle due to highly selected group. However, the allelic frequencies of Karan Fries cattle were almost similar with findings of Huang *et al*. (2010). The reason could be the presence of exotic blood of Holstein Friesian in Karan Fries cattle.

Sequence comparison of three genotypes of lactoferrin gene in Karan Fires and Sahiwal cattle revealed variation at 108th position and T to C transition was observed. Allele B of Lactoferin promoter in Karan Fries and Sahiwal cattle showed cytosine instead of thymine at this position (Figure 1). Similar results were reported by Daly *et al*. (2006) who found 15 Single nucleotide polymorphisms (SNPs) in promoter region of bovine Lactoferrin gene across five cattle breeds including Holstein Friesian. Our results were also in agreement with Seyfert *et al*. (1994). Halloran *et al*., 2009 investigated the SNPs of Lf gene in the Irish bovine population and they reported twenty-nine polymorphisms within a 2.2 kb regulatory region. Nineteen novel polymorphisms were identified and some of these were found within transcription factor binding sites, including GATA-1 and SPI transcription factor sites but none of these SNPs have been verified as a potential genetic marker for production traits in dairy cattle.

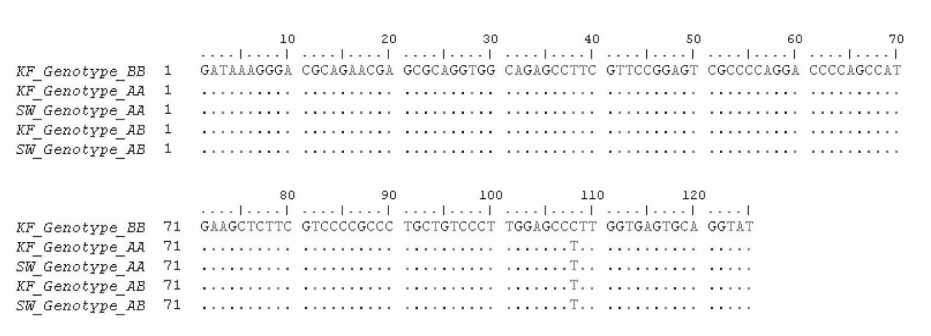

Figure 1: Sequence comparison between different genotypes of lactoferrin promoter in Sahiwal and Karan Fries cattle.

Table: Lactoferrin variants and their association with Clinical Mastitis Incidence

Breed	Lactoferrin genotype	Non- Mastitis cattle	Clinical Mastitis cattle	Mastitis Incidence (%)	Chi Square Value	P – Value
Karan Fries	AA	24	04	14.28	3.913	0.1404^{NS}
	AB	79	36	31.30		
	BB	06	01	14.30		
Sahiwal	AA	59	63	51.63		
	AB	40	38	48.72	0.162	0.6869^{NS}

NS indicate non-significant

Variable incidences of clinical mastitis were observed in the present study. Mastitis incidence was highest for AB genotype followed by BB genotype and lowest in AA genotype in Karan Fries cattle and higher in AA genotype than AB in Sahiwal cattle. The AA genotype in Karan Fries cattle showed lower incidence of clinical mastitis indicating that it is less susceptible to mastitis. It is important to note that heterozygote AB showed lesser incidence of mastitis in Sahiwal. This could be due to absence of BB genotype in the herd leading to skewed frequency. These variable but non-significant results may be due to sampling error, or due to the small sample size, high standard errors and imbalance data. Similar results were reported by Li *et al*., 2004 when they examined the association Lactoferrin promoter with subclinical mastitis. However, Kaminiski *et al*., 2006 reported the polymorphism at +32 position in Lf gene promoter which was significantly associated with protein yield and protein percentage in Polish HF cows. Association of different Lactoferrin allelic variants with incidence of clinical mastitis revealed the non-significant difference (Table) between different genotypes in Karan Fries cattle and two genotypes with clinical mastitis incidence in Sahiwal at 5% level of significance using Chi-square test. The effect of lactoferrin genotype on mastitis incidence in Sahiwal and crossbred Karan Fries cattle only, but this sort of genetic information can be used efficiently in breeding and management decisions in dairy cattle.

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